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Physical Mapping of the Chromosome 7 Breakpoint Region in an SLOS Patient With t(7;20)(q32.1;q13.2)

Tiffany L. Alley,[1] Stephen W. Scherer,[2] Jack J. Huizenga,[2] Lap-Chee Tsui,[2] and Margaret R. Wallace[1*]

[1] *Departments of Pediatrics (Division of Genetics), Biochemistry and Molecular Biology, and the Center for Mammalian Genetics, University of Florida College of Medicine, Gainesville*

[2] *Department of Molecular and Medical Genetics, University of Toronto, and Department of Genetics, The Hospital for Sick Children, Toronto, Canada*

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder characterized by multiple congenital anomalies and mental retardation. SLOS has an associated defect in cholesterol biosynthesis, but the molecular genetic basis of this condition has not yet been elucidated. Previously our group reported a patient with a de novo balanced translocation [t(7;20)(q32.1;q13.2)] fitting the clinical and biochemical profile of SLOS. Employing fluorescence in situ hybridization (FISH), a 1.8 Mb chromosome 7-specific yeast artificial chromosome (YAC) was identified which spanned the translocation breakpoint in the reported patient. The following is an update of the on-going pursuit to physically and genetically map the region further, as well as the establishment of candidate genes in the 7q32.1 breakpoint region. Am. J. Med Genet. 68:279–281, 1997.

KEY WORDS: Smith-Lemli-Opitz syndrome (SLOS); positional cloning; balanced translocation; physical map; chromosome 7

INTRODUCTION

We previously reported a de novo translocation, [t(7;20)(q32.1;q13.2)], in a patient, UF53, clinically diagnosed with the autosomal recessive disorder Smith-Lemli-Opitz syndrome (SLOS) [Wallace et al., 1994]. This patient and reports of other SLOS patients [Curry et al., 1987; Berry et al., 1989] with chromosome rearrangements of the 7q region suggest that a causative gene for the disease might be located there. SLOS displays variable expression of congenital anomalies and mental retardation. Most affected individuals have significantly low serum and tissue cholesterol levels and a 200–2,000-fold elevation in the penultimate precursor, 7-dehydrocholesterol [Tint et al., 1995]. We confirmed the biochemical diagnosis of SLOS in UF53 [Alley et al., 1995]. In an attempt to elucidate the causative nature of SLOS, our group is using positional cloning techniques to identify putative gene(s) in the 7q32.1 region disrupted by UF53's translocation. The following is an update of the data introduced at the September 1995 NICHD Meeting on the RSH/SLO syndrome.

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*Correspondence to: Dr. Margaret R. Wallace, Division of Genetics, Department of Pediatrics, University of Florida Health Science Center, P.O. Box 100296, Gainesville, FL 32610-0296.

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RESULTS

Refined Localization of the Translocation Breakpoint at 7q32.1 Using FISH

Previously we identified a set of overlapping yeast artificial chromosome (YAC) clones spanning the 7q32.1 translocation breakpoint in UF53 [Alley et al., 1995]. Since that report, the physical map of the region has been refined by mapping DNA probes, cosmids and P1-artificial chromosomes (PACs) [Ioannou et al., 1994] specific to this region. Also, a rare-enzyme restriction map of the YACs has been constructed (described below; Fig. 1). Using fluorescence in situ hybridization (FISH) analysis, two cosmids, 85d12 and 153a8, were positioned directly proximal and distal, respectively, to the translocation breakpoint. The two cosmids are separated by approximately 100–200 kb (Fig. 1). Currently, FISH analysis is underway to position the PACs relative to the breakpoint; the PACs were initially anchored on the map using microsatellite markers from the region [Alley et al., 1995]. Since the PACs contain an average insert size of approximately 130 kb, it is hoped that one or more of these clones will span the breakpoint to further focus efforts.

Rare Restriction Site Map of the 7q32.1

Using restriction enzymes that recognize CpG islands (or HTF islands) along with pulsed-field gel electrophoresis (PFGE), a map of the YAC clones surrounding the translocation breakpoint was constructed (Fig. 1). CpG islands tend to be found in the 5′ unmethylated

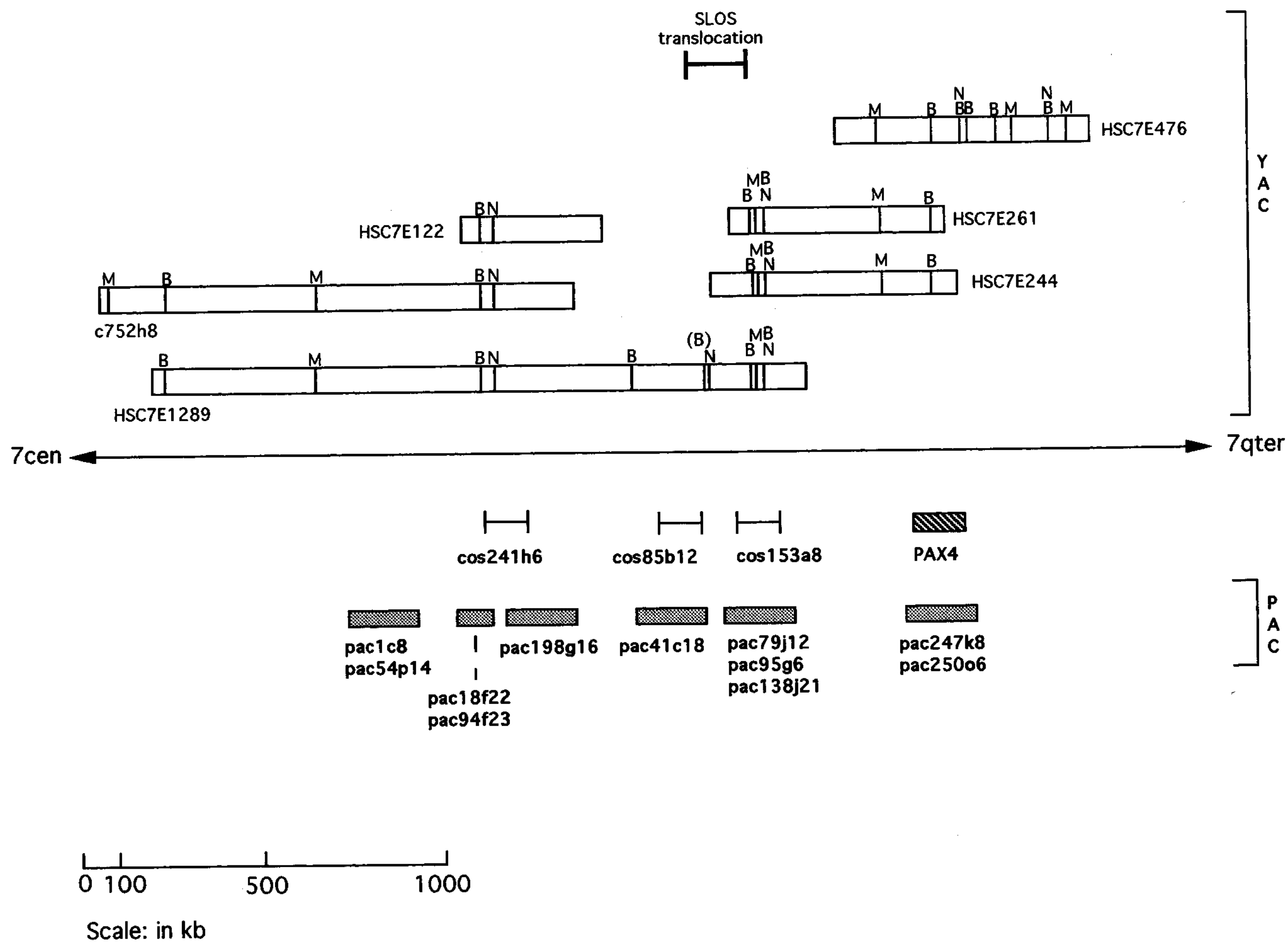

Fig. 1. Schematic map of the translocation region at 7q32.1. The YACs (open boxes) are to scale and depict the rare-restriction sites established from PFGE analysis: B=BssHII, N=NotI, and M=MluI. The cosmids (indicated by "cos") and the *PAX4* gene were placed on the map by hybridization of a mouse cDNA probe (kindly provided by Peter Gruss, Max Planck Institute, Germany) against Southern blots of the YACs. The PACs (stippled boxes) are positioned with respect to their cosmid counterpart and microsatellite markers. The cosmids, hatched and stippled boxes are not to scale. The location of the t(7;20) breakpoint is identified by the bracketed region, between cosmids 85b12 and 153a8. The complete distribution of the *GMR8* gene on HSC7E1289 is currently unknown and is thus not indicated on this map.

GC-rich regions of genes [Bird, 1987]. A CpG island consisting of 5 rare-enzyme restriction sites (one BssHII site, three MluI sites, and one NotI site) was identified distal to the translocation. The cosmid clone 153a8 was also shown to contain the identical cluster of restriction enzyme sites, allowing it to be aligned to the YAC contig. Additional restriction sites were identified allowing further YAC alignment.

DISCUSSION

This de novo balanced translocation represents a valuable resource in the positional cloning of a gene involved in SLOS, under the hypothesis that the breakpoint disrupts an SLOS gene is true. Since our last report we have been able to narrow the precise location of the breakpoint from a megabase-interval to a region of less than 200 kb. We are currently conducting chromosome walking experiments using cosmids and PACs in order to identify a genomic clone that encompasses the breakpoint. PFGE mapping demonstrated the existence of an apparent CpG island on the YAC contig near the distal end of HSC7E1289 that was confirmed to lie in cosmid 153a8. Since this cosmid is directly adjacent to the translocation breakpoint, the identification of a gene associated with this CpG island could be relevant in SLOS.

Candidate gene isolation experiments using direct cDNA selection, searching for DNA sequences conserved in evolution, and further identification of CpG islands are being conducted; one candidate gene on HSC7E1289 has thus far been identified. *GMR8* encodes a metabotropic glutamate receptor, one of a family of such genes located throughout the genome. This partial cDNA was recently found to lie on HSC7E1289; however, the genomic structure, sequence, and expression pattern of this gene has not yet been determined

[Scherer et al., 1996]. While a partial *GMR8* cDNA probe clearly lies centromeric to the breakpoint [Scherer et al., 1996], it is unknown whether the CpG island in cosmid 153a8 corresponds to the 5′ end of *GMR8*. Thus, it is not clear whether or not this gene might be disrupted by the breakpoint or involved in SLOS. Experiments to answer these questions are underway. Although this gene is not known to be involved in cholesterol synthesis, it is still unclear if its disrupted function could contribute to the phenotype.

Of additional interest is the recent mapping of the *PAX4* gene to the same general region as the translocation breakpoint. *PAX4* is one of nine genes belonging to the *PAX* family of homeobox genes. This family encodes nuclear transcription factors involved in developmental control during embryogenesis in both vertebrates and invertebrates [Stuart and Gruss, 1995]. *PAX4* was regionally mapped to the chromosome band 7q32 by FISH [Tamura et al., 1994], and we have positioned the *PAX4* gene within 500 kb telomeric of the breakpoint. Owing to its relatively close proximity to the translocation breakpoint and its ubiquitous involvement in transcriptional regulation during embryogenesis, we are currently investigating its possible involvement in SLOS. It is conceivable that the expression of this gene (or others) could be influenced by a positional effect similar to that described in campomelic dysplasia [Wagner et al., 1994].

In addition to positional cloning of the translocation breakpoint, our group is also using genetic linkage analysis to confirm that this region is involved in SLOS and to determine if this disease displays genetic heterogeneity. Toward this end, we are collecting and genotyping families with multiple affected sibs or with multiple branches of the pedigree affected. Contributions of additional family samples for this project (or other patients with chromosome rearrangements) would be greatly appreciated; interested individuals should contact the corresponding author.

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